

Figure 1. Baseline clinical parameters (mean \pm SD) in both mild to moderate (NYHA I-II) and severe (NYHA III-IV) congestive heart failure (CHF) groups.

	Mild to Moderate CHF n = 40	Severe CHF n = 47
Age (years)	62 \pm 7	67 \pm 8 §
Ejection fraction (%)	29 \pm 4	20 \pm 6 §§
Gender (Male/Female)	36/4	38/9
Ischemic (n)	38	38
Non-ischemic (n)	2	9

§ p<0.01, §§ p<0.001 severe vs. mild to moderate CHF

Figure 2.
Kaplan-Meier survival curves in patients with mild to moderate (NYHA Class I-II) and severe (NYHA Class III-IV) congestive heart failure (CHF)

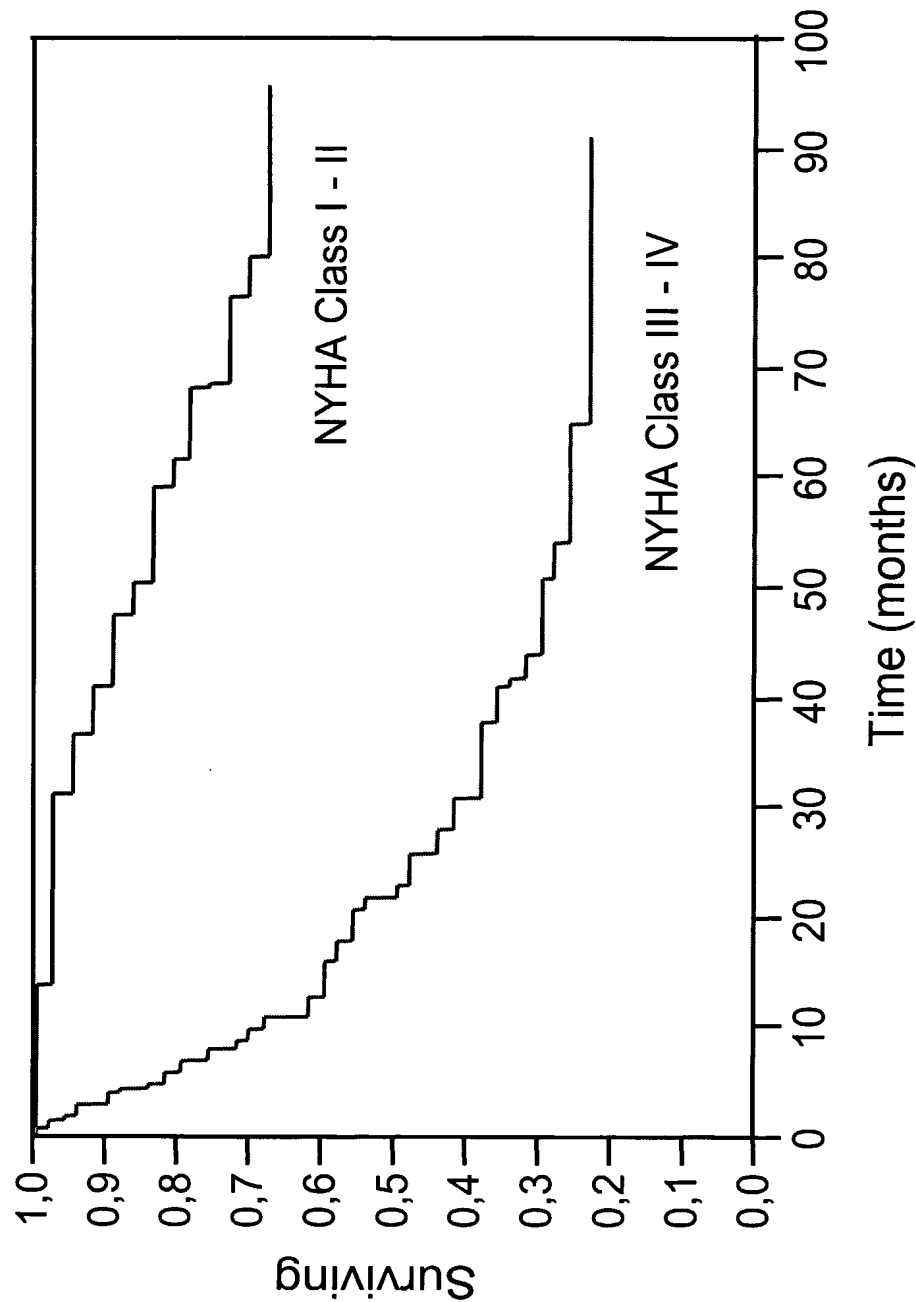


Figure 3. Values for the natriuretic and vasoactive peptides in healthy control individuals

Parameter	Method	Specificity	Units	n	Geometrical mean	99.5 % Confidence Limits of the mean	
						Low limit	High limit
Big-ET-1 (1-38)	ELISA (2-site)	C-proET-1 (1-38)	fmol/ml	50	0.96	0.81	1.16
Big-ET-1 (22-38)	RIA	C-proET-1 (1-38) and fragments of Big-ET-1 with epitopes between (22-38)	pg/ml	92	5.5	5.3	5.8
ET-1	RIA	ET-1 (1-21) and peptides with epitopes between (1-21)	pg/ml	92	4.1	3.8	4.4
N-proANP (1-98)	ELISA (2-site)	N-proANP (1-98)	fmol/ml	60	1692	1466	1952
N-proANP (1-25)	RIA	N-proANP (1-98) and peptides with epitopes between (1-25)	pg/ml	92	167	149	188
N-proANP (68-98)	RIA	N-proANP (1-98) and peptides with epitopes between (68-98)	pg/ml	92	268	236	303
BNP	IRMA (2-site)	BNP (77-108)	pg/ml	28	9	5	15
BNP	RIA	BNP (77-108) and peptides with epitopes between 77-108 of N-proBNP	pg/ml	92	5.0	4.4	5.6
N-proBNP	ELISA (Competition)	BNP (1-76) and peptides with epitopes between (1-76)	fmol/ml	50	267	224	319

Figure 4. Baseline neurohormonal data [geometric mean (range)] in both mild to moderate and severe CHF groups, and comparison with healthy controls.

	Controls ^{φφ}	Mild to Moderate CHF n = 40	Severe CHF n = 47
N-proANP (1-98) fmol/ml	1692 (212-3908)	3192 (1001-7768)*	5528 (1129-18180)**§§
N-proANP (1-25) pg/ml	167 (66-354)	537 (145-2010)	1903 (623-7148)**§§
N-proANP (68-98) pg/ml	268 (94-898)	860 (164-2480)*	2507 (709-9075)**§§
N-proBNP fmol/ml	267 (95-1097)	491 (109-1610)	1521 (598-5491)**§§
BNP IRMA pg/ml	9 (2-49)	43 (3-212)	328 (28-1610)**§
BNP RIA pg/ml	5 (3-15)	22 (10-64)*	75 (15-192)**§§
ET-1 pg/ml	4.1 (2.1-6.6)	3.5 (0.9-8.3)	10.0 (8.7-11.6)**§§
BIG ET-1 RIA pg/ml	5.5 (4.0-7.6)	7.2 (3.8-12.2)	12.0 (5.8-22.9)**§§
BIG ET-1 ELISA fmol/ml	1.0 (0.4-2.8)	1.8 (0.6-4.5)	3.8 (1.3-14.5)**§§

*p<0.05, ** p<0.001 vs controls

§ p<0.01, §§ p<0.001 severe vs mild to moderate CHF

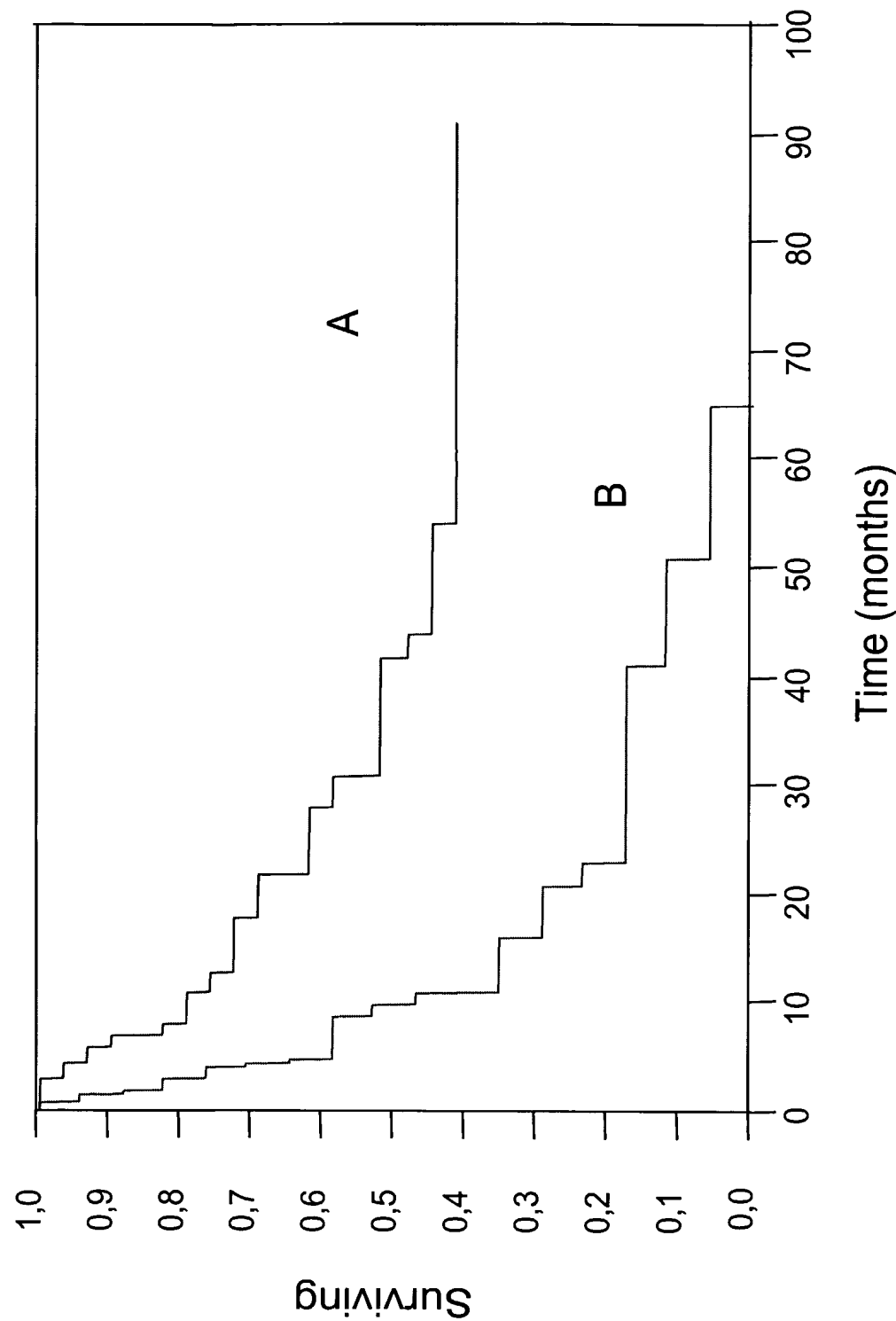
^{φφ} Data derived from Figure 3

Figure 5. Prognostic values for survival using natriuretic and vasoactive peptide assays, considered independently in NYHA III-IV patients *

Prognostic parameters considered independently			Best estimate of cutoff level segregating the patients with good and poor survival prognosis				Median survival time estimation (months)					
			Value of the cutoff level	Cutoff levels expressed as fold geometric mean of normal	Cutoff levels expressed as fold 99.5% Confidence Limits of the geometrical mean					Bad prognosis group	Good Prognosis group	Test between groups Prob>ChiSq Wilcoxon
Big-ET-1 (1-38)	2-site ELISA	fmol/ml	4.0	4.1	3.5	5.0	10	31	0.0005			
Big-ET-1 (22-38)	RIA	pg/ml	13.8	2.5	2.4	2.6	10	30	0.0255			
ET-1	In-house RIA	pg/ml	8.4	2.1	1.9	2.2	9	30	0.0149			
N-proANP (1-98)	2-site ELISA	fmol/ml	6483	3.8	3.3	4.4	10	43	0.003			
N-proANP (1-25)	In-house RIA	pg/ml	1784	10.7	9.5	12.0	13	44	0.0204			
N-proANP (68-98)	In-house RIA	pg/ml	2562	9.6	8.5	10.8	11	31	0.0092			
BNP	IRMA	pg/ml	480	54	32	89	10	28	0.1238			
BNP	In-house RIA	pg/ml	92	18	16	21	9	28	0.0577			
N-proBNP	Competition-ELISA	fmol/ml	1512	5.7	4.7	6.8	11	42	0.0423			

* The specificity of each assay is summarized in Figure 3 and described in full length in the text.

Figure 6. Patients with severe CHF (NYHA Class III-IV)
A : Big ET-1 (1-38) <4.0 pg/ml
B : Big ET-1 (1-38) >4.0 pg/ml



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Figure 7. Patients with severe CHF (NYHA Class III-IV)

A : N-pro ANP (1-98) <6483 fmol/ml

B : N-pro ANP (1-98) >6483 fmol/ml

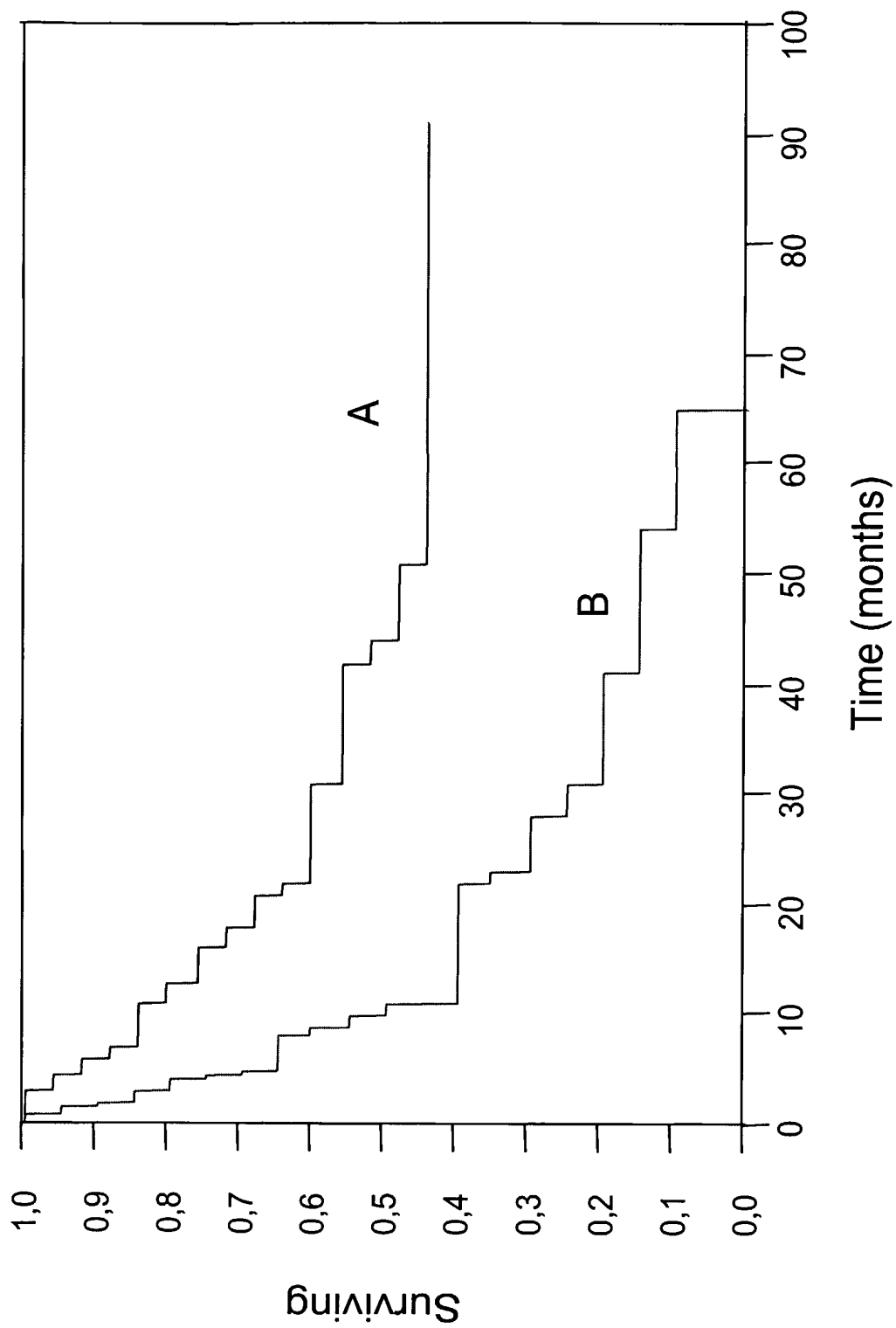
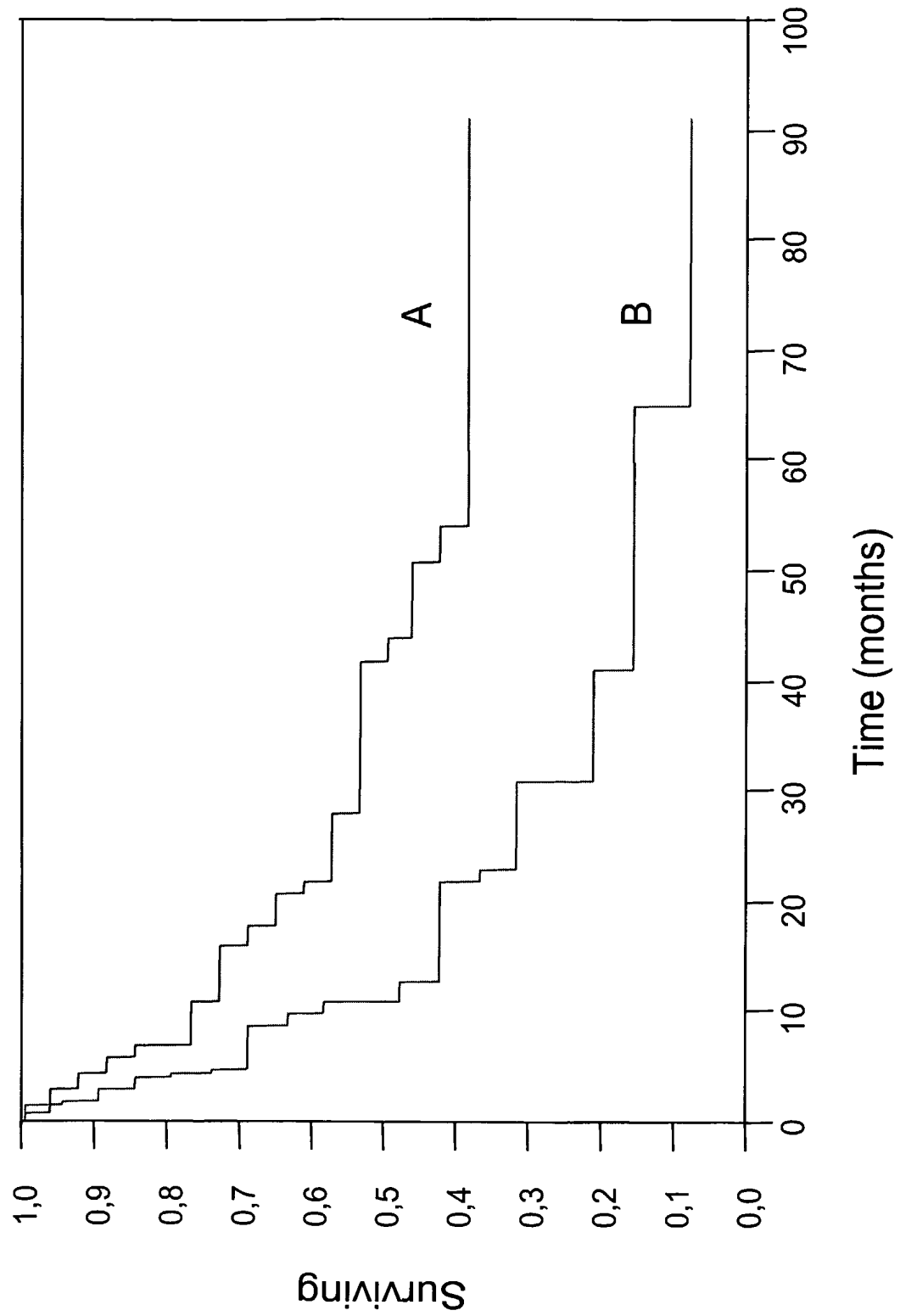


Figure 8. Patients with severe CHF (NYHA Class III-IV)

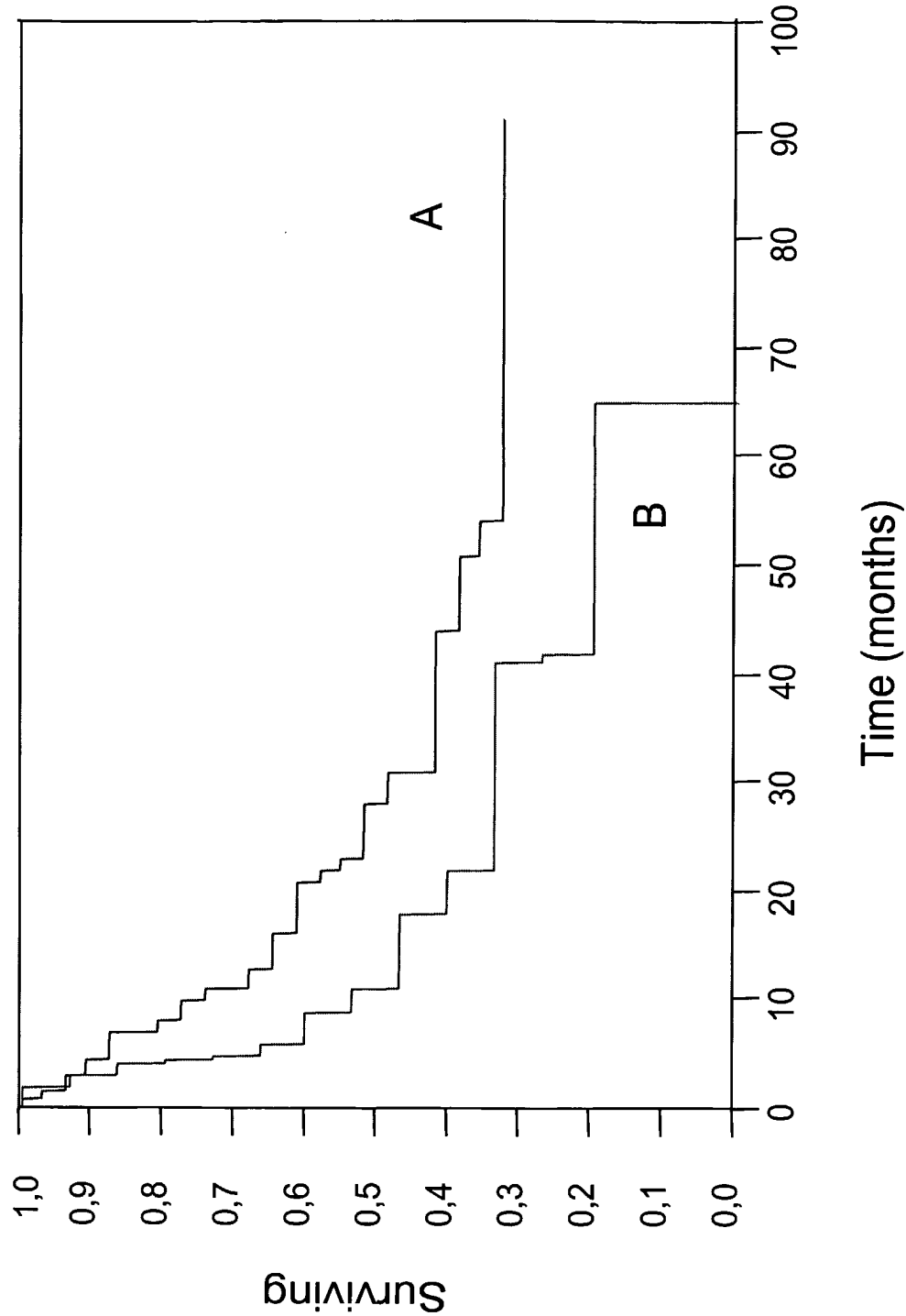
A : N-proBNP <1512 fmol/ml

B : N-proBNP >1512 fmol/ml



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Figure 9. Patients with severe CHF (NYHA Class III-IV)
A : BNP (IRMA) <480 pg/ml
B : BNP (IRMA) >480 pg/ml



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Figure 10. Prognostic values for survival using Big-ET-1 (1-38) testing determined with a specific 2 site ELISA, used in combination with several other natriuretic and vasoactive peptide assays in NYHA III-IV patients *

Parameters considered in combination		Classification based on cut-off combinations (Cutoff levels are as in Figure 5) L : marker below cut-off H : marker above cut-off	Estimation of 50 % survival in months	Number of deaths during study period	Survivors at end of study period (censored)	Total Number/Class	Total	Test between groups Prob>ChiSq Wilcoxon
Marker 1 (M1)	Marker 2 (M2)							
Big ET-1 (1-38) (2-site ELISA)	N-proANP (2-site ELISA)	M1 L and M2 L	> 91	10	11	21	45	0.0002
		M1 H and M2 L OR M1 L and M2 H	22	10	1	11		
		M1 H and M2 H	5	13	0	13		
Big ET-1 (1-38) (2-site ELISA)	N-proANP (1-25) (RIA)	M1 L and M2 L	60	11	11	22	46	0.0013
		M1 H and M2 L OR M1 L and M2 H	16	11	1	12		
		M1 H and M2 H	5	12	0	12		
Big ET-1 (1-38) (2-site ELISA)	N-proANP (68-98) (RIA)	M1 L and M2 L	>91	8	10	18	46	0.0003
		M1 H and M2 L OR M1 L and M2 H	21	14	2	16		
		M1 H and M2 H	4.5	12	0	12		

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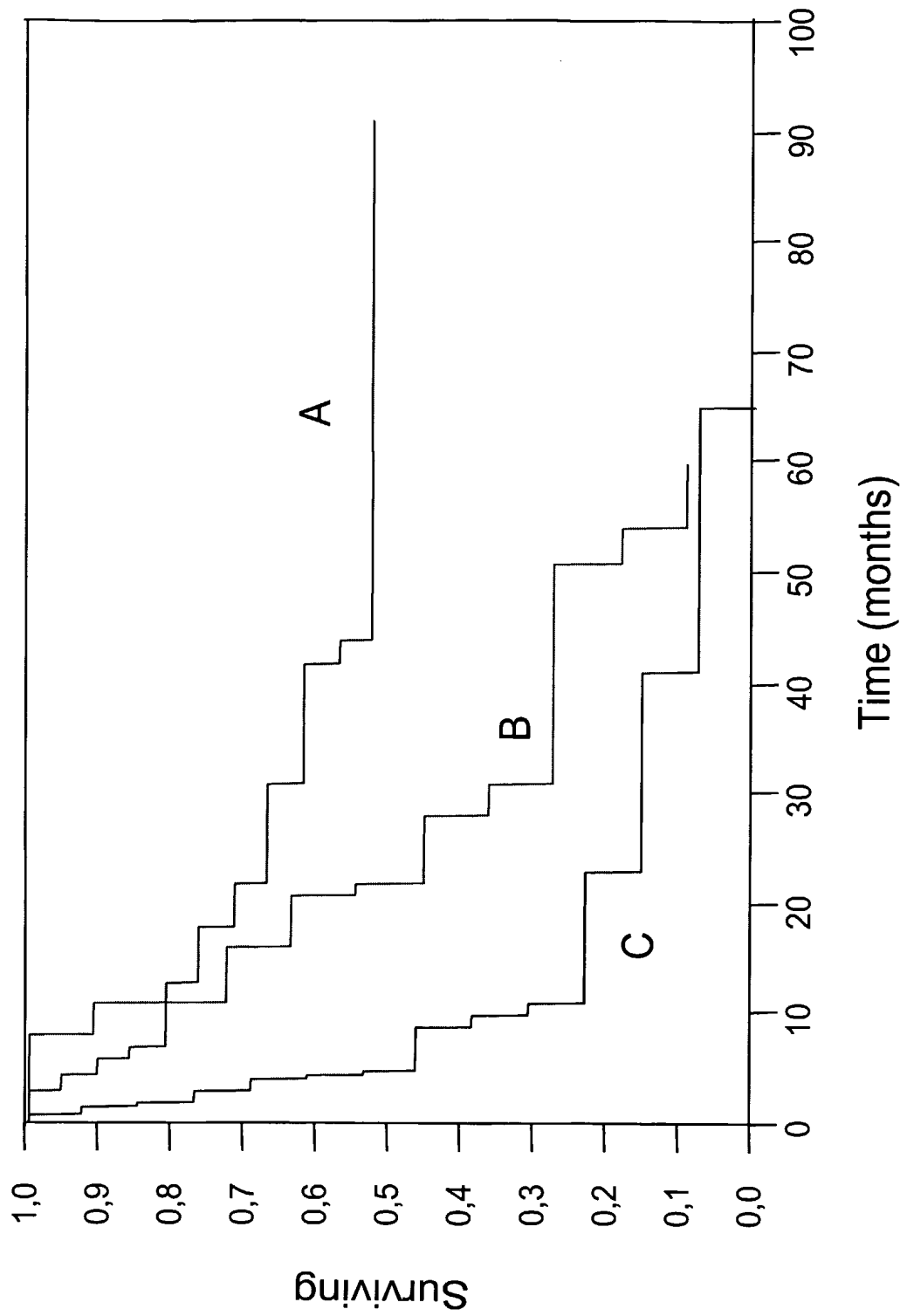
Parameters considered in combination		Classification based on cut-off combinations (Cutoff levels are as in Figure 5) L : marker below cut-off H : marker above cut-off	Estimation of 50 % survival in months	Number of deaths during study period	Survivors at end of study period (censored)	Total Number/Class	Total	Test between groups Prob>ChiSq Wilcoxon
Marker 1 (M1)	Marker 2 (M2)							
Big ET-1 (1-38) (2-site ELISA)	N-proBNP Competition one site ELISA	M1 L and M2 L	61	9	9	18	41	0.0009
		M1 H and M2 L OR M1 L and M2 H	22	8	2	10		
		M1 H and M2 H	7.5	13	0	13		
Big ET-1 (1-38) (2-site ELISA)	BNP (IRMA)	M1 L and M2 L	44	12	10	22	46	0.0044
		M1 H and M2 L OR M1 L and M2 H	16	14	2	16		
		M1 H and M2 H	4.5	8	0	8		
Big ET-1 (1-38) (2-site ELISA)	BNP (RIA)	M1 L and M2 L	31	13	8	21	46	0.0011
		M1 H and M2 L OR M1 L and M2 H	20	11	4	15		
		M1 H and M2 H	4.5	10	0	10		

Parameters considered in combination		Classification based on cut-off combinations (Cutoff levels are as in Figure 5) L : marker below cut-off H : marker above cut-off	Estimation of 50 % survival in months	Number of deaths during study period	Survivors at end of study period (censored)	Total Number/Class	Total	Test between groups Prob>ChiSq Wilcoxon
Marker 1 (M1)	Marker 2 (M2)	M1 L and M2 L	37	10	7	17	36	0.0055
Big ET-1 (1-38) (2-site ELISA)	ET-1 (RIA)	M1 H and M2 L OR M1 L and M2 H	12	10	1	11		
		M1 H and M2 H	4.5	8	0	8		

* The specificity of each assay is summarized in Figure 3 and described in full length in the text.

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Figure 11. Patients with severe CHF (NYHA Class III-IV)
A : Big-ET-1 (1-38) <4.0 pg/ml AND N-proANP (1-98) <6483 fmol/ml
B : Big-ET-1 (1-38) >4.0 pg/ml OR N-proANP (1-98) >6483 fmol/ml
C : Big-ET-1 (1-38) >4.0 pg/ml AND N-proANP (1-98) >6483 fmol/ml



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Figure 12. Patients with severe CHF (NYHA Class III-IV)
A : Big ET-1 (1-38) <4.0 pg/ml AND N-proANP (1-98) <6483 fmol/ml
B : Big ET-1 (1-38) >4.0 pg/ml AND N-proANP (1-98) >6483 fmol/ml

Solid lines: survival

Dotted lines: confidence limits

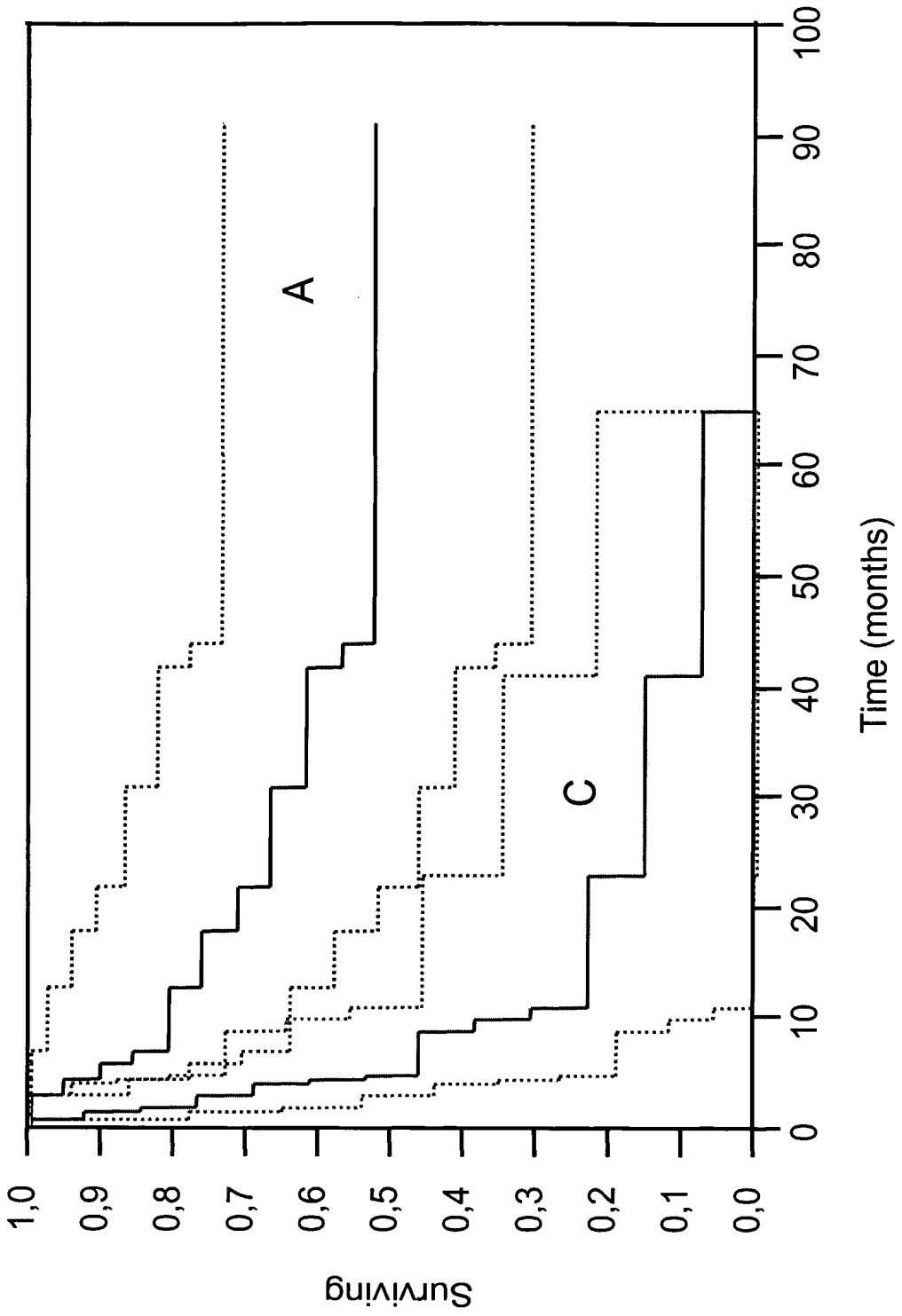


Figure 13. Prognostic values for survival using N-proBNP testing determined with a single site competition ELISA used in combination with 2 different BNP assays in NYHA III-IV patients *

Parameters considered in combination		Classification based on cut-off combinations (Cut-off levels are as in Fig. 5) L : below cut-off H : above cut-off	Estimation of 50 % survival in months	Number of deaths during study period	Survivors at end of study period (censored)	Total Number/ Class	Total	Test between groups Prob>ChiSq Wilcoxon
Marker 1	Marker 2							
N-proBNP Competition one site ELISA	BNP IRMA	M1 L and M2 L	44	12	8	20	45	0.0282
		M1 H and M2 L OR M1 L and M2 H	18	12	4	16		
		M1 H and M2 H	5	9	0	9		
N-proBNP Competition one site ELISA	BNP RIA	M1 L and M2 L	44	11	8	19	45	0.0343
		M1 H and M2 L OR M1 L and M2 H	18	13	3	16		
		M1 H and M2 H	5	9	1	10		

* The specificity of each assay is summarized in Figure 3 and described in full length in the text.

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Figure 14. Patients with severe CHF (NYHA Class III-IV)
A : N-proBNP <1512 fmol/ml AND BNP (IRMA) <480 pg/ml
B : N-proBNP >1512 fmol/ml OR BNP (IRMA) >480 pg/ml
C: N-proBNP >1512 fmol/ml AND BNP (IRMA) >480 pg/ml

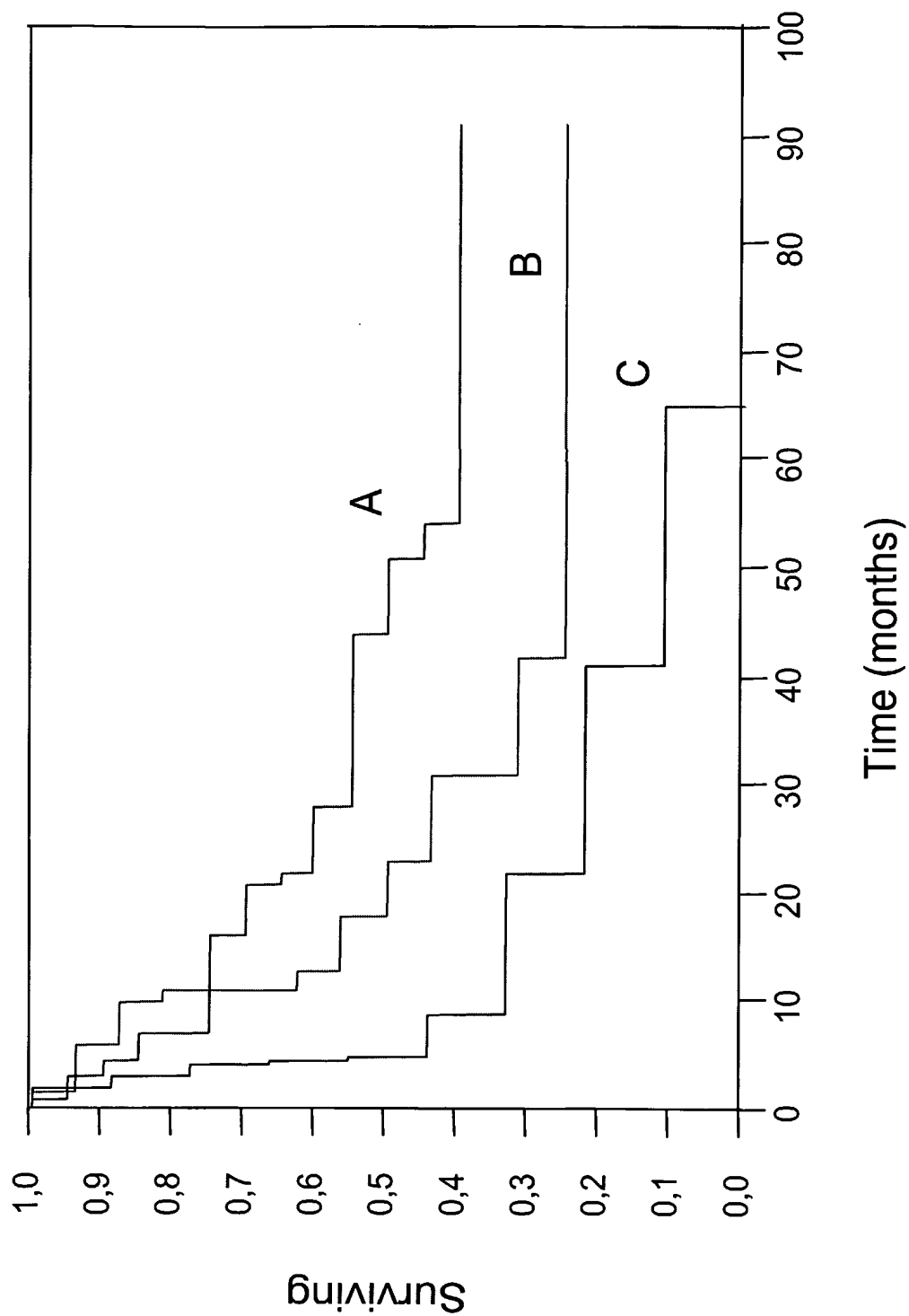


Figure 15. Patients with severe CHF (NYHA Class III - IV)
A : N-proBNP <1512 fmol/ml AND BNP (IRMA) <480 pg/ml
C: N-proBNP >1512 fmol/ml AND BNP (IRMA) >480 pg/ml
Solid lines: survival
Doted lines: confidence limits

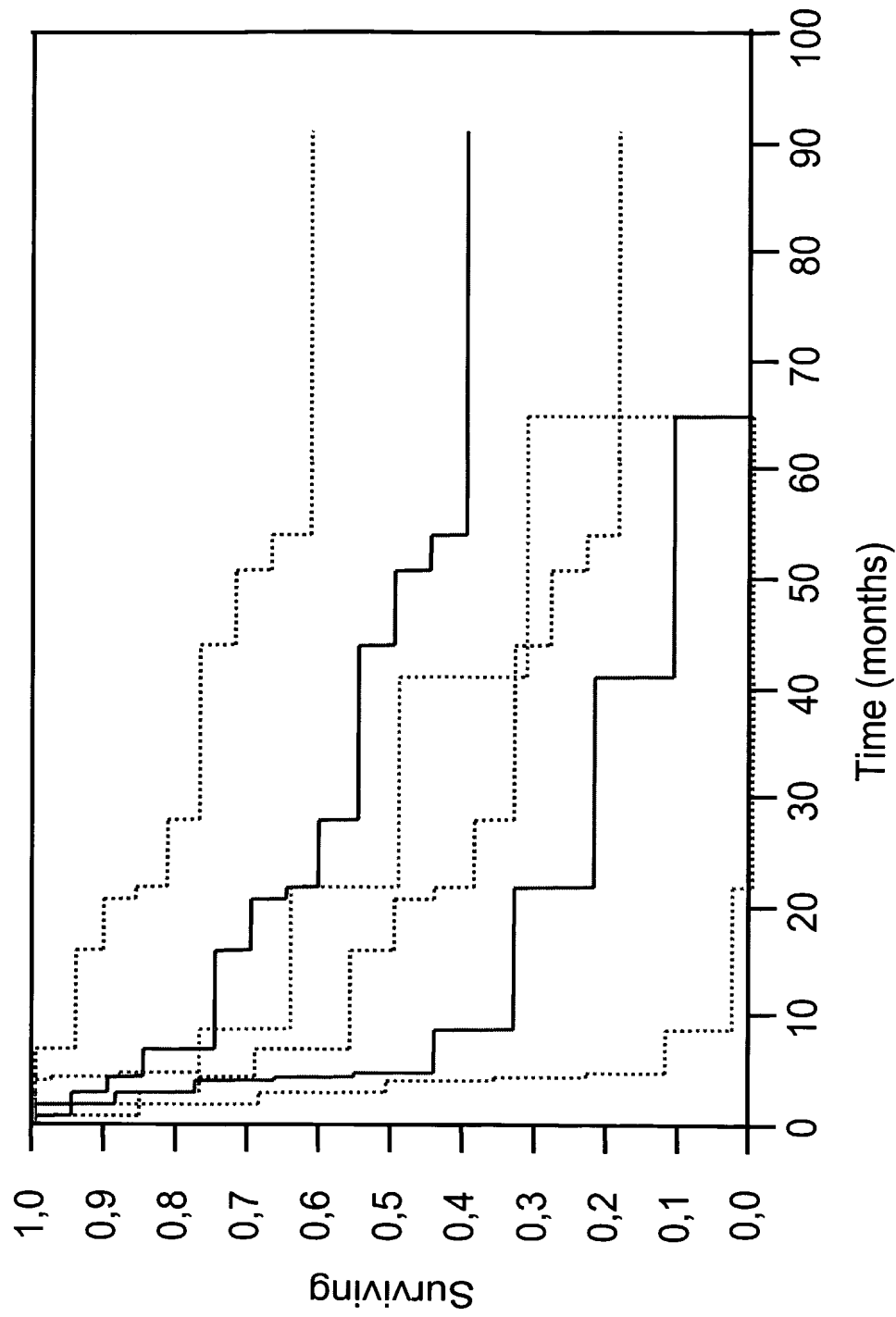


Figure 16. Prognostic values for survival using several natriuretic and vasoactive peptide assays, considered independently in NYHA I - II patients *

Prognostic parameters considered independently	Best estimate of cutoff level segregating patients with good and poor survival prognosis			Classification based on cut-off L : below cut-off H : above cut-off	75% survival estimation (months)	% Survival at end of study	Number of deaths during study period	Survivors at end of study period (censored)	Total Number/ Class	Test between groups Prob>ChiSq Wilcoxon
	Value of the cutoff level	Cutoff levels expressed as fold geometrical mean of normal	Cutoff levels expressed as fold 99.5% Confidence Limits of the geometrical mean							
N-proANP (1-98)	2-site ELISA	3292	1.9	L	>91	84	3	16	19	0.0107
N-proANP (1-25)	In-house RIA	496	3.0	L	>91	79	4	15	19	0.0772
N-proANP (68-98)	In-house RIA	736	2.8	L	>91	87	2	13	15	0.0474
BNP	IRMA	38	4.2	L	>91	81	3	13	16	0.1078
BNP	In-house RIA	25	5.0	L	>91	81	4	18	22	0.0178
				H	47	57	8	7	15	

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Prognostic parameters considered independently			Best estimate of cutoff level segregating patients with good and poor survival prognosis			Classification based on cut-off L : below cut-off H : above cut-off	75% survival estimation (months)	% Survival at end of study	Number of deaths during study period	Survivors at end of study period (censored)	Total Number/ Class	Test between groups Prob>ChiSq Wilcoxon
N-proBNP	Competition-ELISA	fmol/ml	Value of the cutoff level	Cutoff levels expressed as fold geometric mean of normal	Cutoff levels expressed as fold 99.5% Confidence Limits of the geometrical mean							
			404	1.5	1.3 1.8	L	>91	86	2	12	14	0.0755
						H	53	56	10	13	23	
Big-ET-1 (1-38)	2-site ELISA	fmol/ml	1.9	2.0	1.6 2.4	L	>91	79	5	19	24	0.0204
						H	39	46	7	6	13	
Big-ET-1 (22-38)	RIA	pg/ml	--	--	--	L	--	--	--	--	--	0.1441
						H	--	--	--	--	--	
ET-1	In-house RIA	pg/ml	--	--	--	L	--	--	--	--	--	0.2620
						H	--	--	--	--	--	

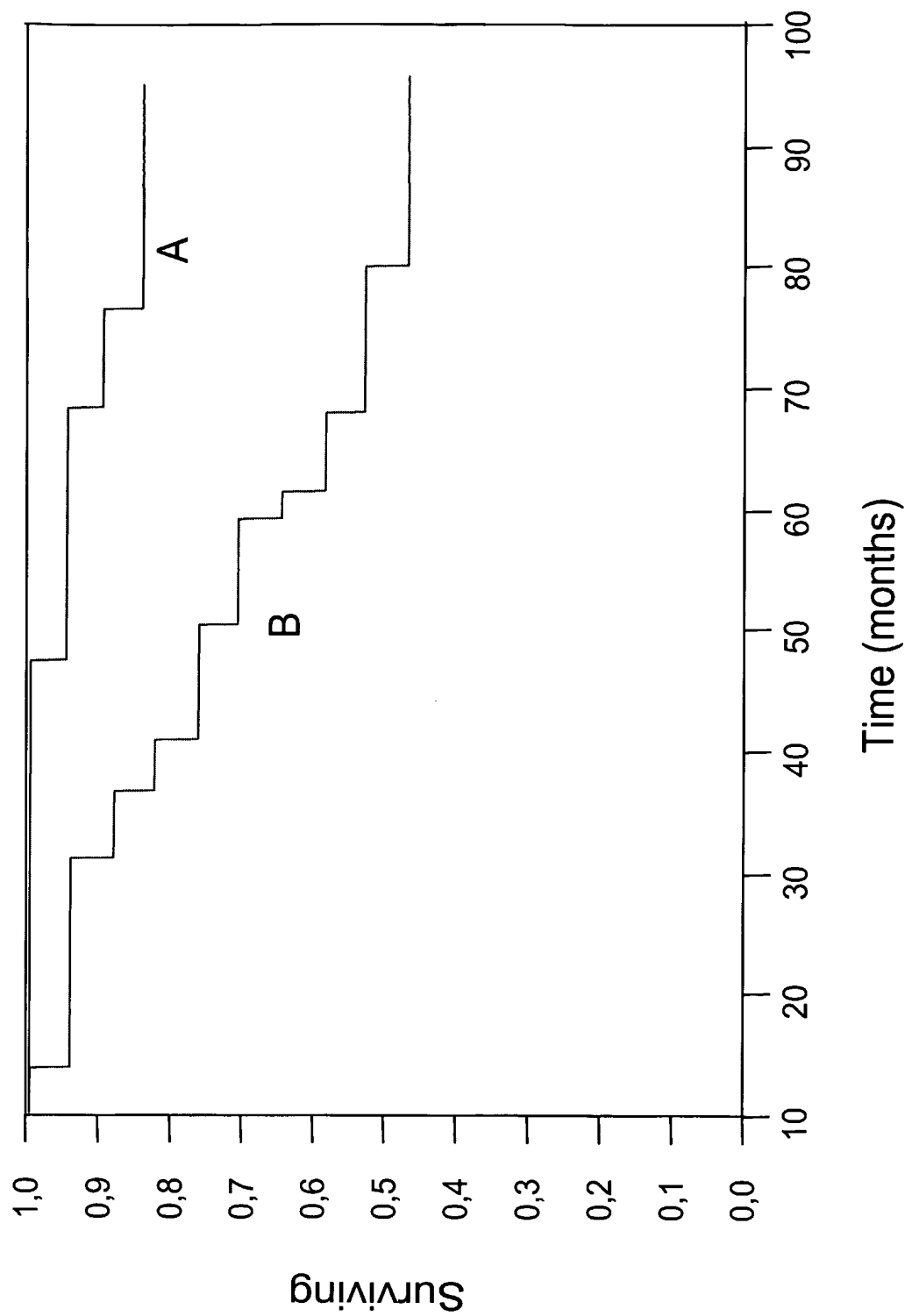
* The specificity of each assay is summarized in Figure 3 and described in full length in the text.

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Figure 17. Patients with mild to moderate CHF (NYHA Class I-II)

A : N-proANP (1-98) < 3292 fmol/ml

B : N-proANP (1-98) > 3292 fmol/ml



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Figure 18. Patients with mild to moderate CHF (NYHA Class I-II)

A : N-proBNP < 404 fmol/ml

B : N-proBNP > 404 fmol/ml

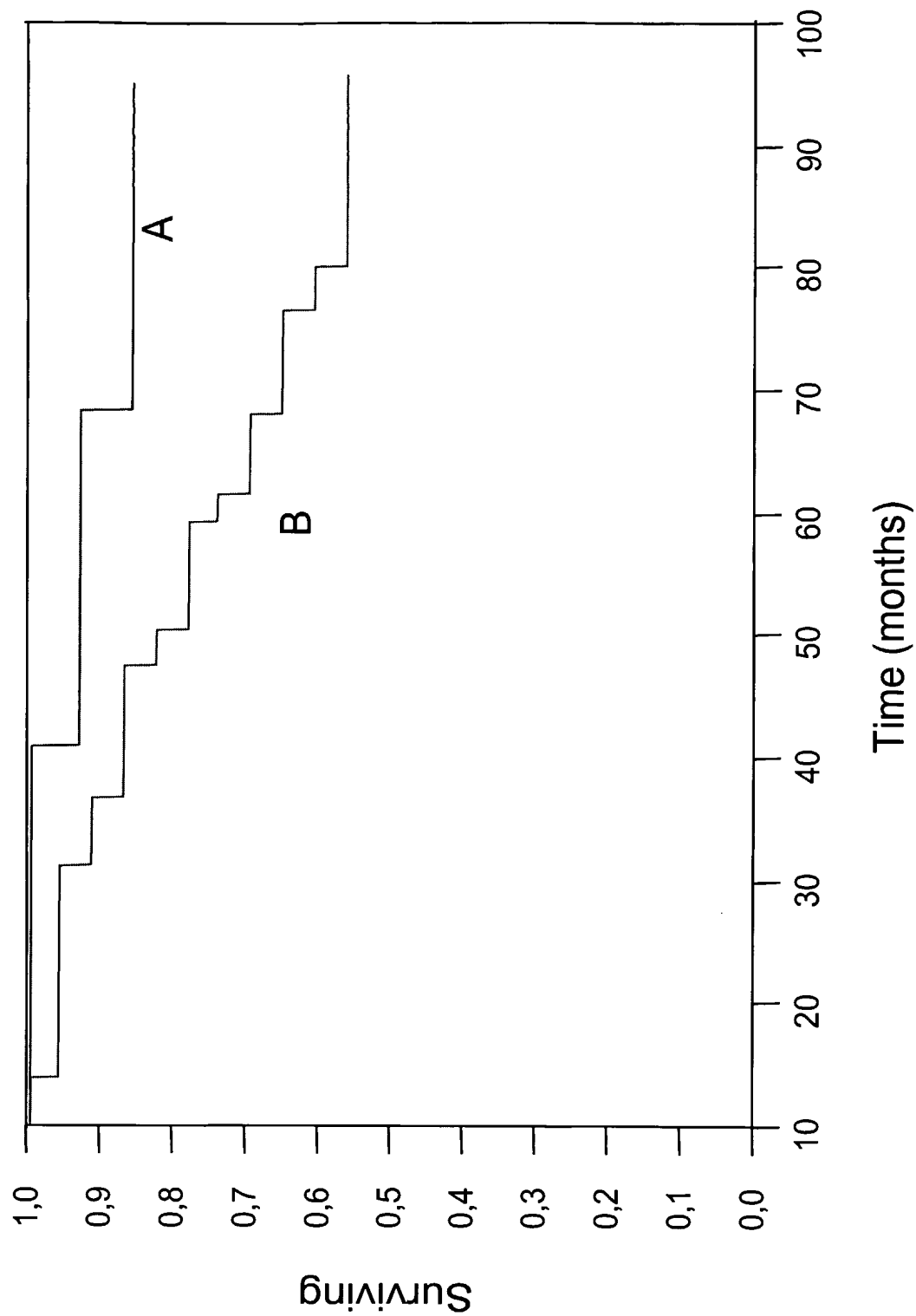


Figure 19. Patients with mild to moderate CHF (NYHA Class I-II)

A : BNP (RIA) < 25 pg/ml

B : BNP (RIA) > 25 pg/ml

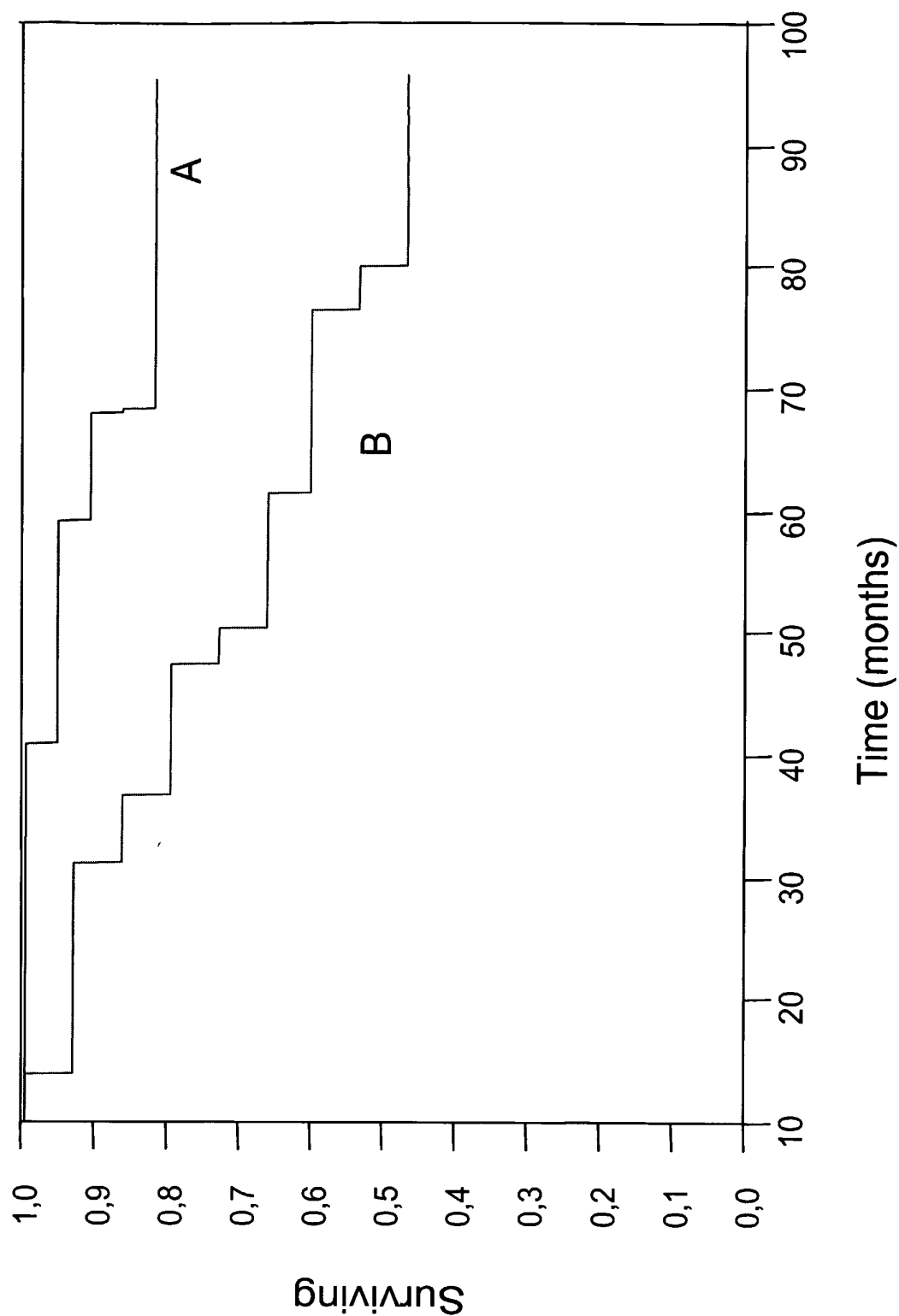


Figure 20. Prognostic values for survival using N-proANP (1-98) testing determined with a specific 2-site ELISA, used in combination with several other natriuretic and vasoactive peptide assays in NYHA I-II patients *

Parameters considered in combination		Classification based on cut-off combinations (Cut-off levels are as in Fig 5) L : below cut-off H : above cut-off	% Survival at end of study	75% survival estimation (months)	Number of deaths during study period	Survivors at end of study period (censored)	Total Number/ Class	Total	Test between groups Prob>ChiSq Wilcoxon
Marker 1 (M1)	Marker 2 (M2)								
N-proANP (1-98) (2-site ELISA)	BNP (IRMA)	M1 L and M2 L	92	>91	1	11	12	36	0.0596
		M1 H and M2 L OR M1 L and M2 H	60	70	4	6	10		
		M1 H and M2 H	50	42	7	7	14		
N-proANP (1-98) (2-site ELISA)	BNP (RIA)	M1 L and M2 L	93	>91	1	13	14	36	0.0114
		M1 H and M2 L OR M1 L and M2 H	58	59	5	7	12		
		M1 H and M2 H	40	35	6	4	10		

Parameters considered in combination		Classification based on cut-off combinations (Cut-off levels are as in Fig 5) L : below cut-off H : above cut-off	% Survival at end of study	75% survival estimation (months)	Number of deaths during study period	Survivors at end of study period (censored)	Total Number/ Class	Total	Test between groups Prob>ChiSq Wilcoxon
Marker 1 (M1)	Marker 2 (M2)								
N-proANP (1-98) (2-site ELISA)	N-proBNP Competition one site ELISA	M1 L and M2 L	92	>91	1	11	12	36	0.0417
		M1 H and M2 L OR M1 L and M2 H	67	50	3	6	9		
		M1 H and M2 H	47	40	8	7	15		
N-proANP (1-98) (2-site ELISA)	Big ET-1 (2-site ELISA)	M1 L and M2 L	84	>91	3	16	19	36	0.0365
		M1 H and M2 L OR M1 L and M2 H	50	41	2	2	4		
		M1 H and M2 H	46	40	7	6	13		

* The specificity of each assay is summarized in Figure 3 and described in full length in the text.

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Figure 21. Patients with mild to moderate CHF(NYHA Class I-II)
A : N-proANP (1-98) < 3292 fmol/ml AND N-proBNP < 404 fmol/ml
B : N-proANP (1-98) > 3292 fmol/ml OR N-proBNP > 404 fmol/ml
C: N-proANP (1-98) > 3292 fmol/ml AND N-proBNP >404 fmol/ml

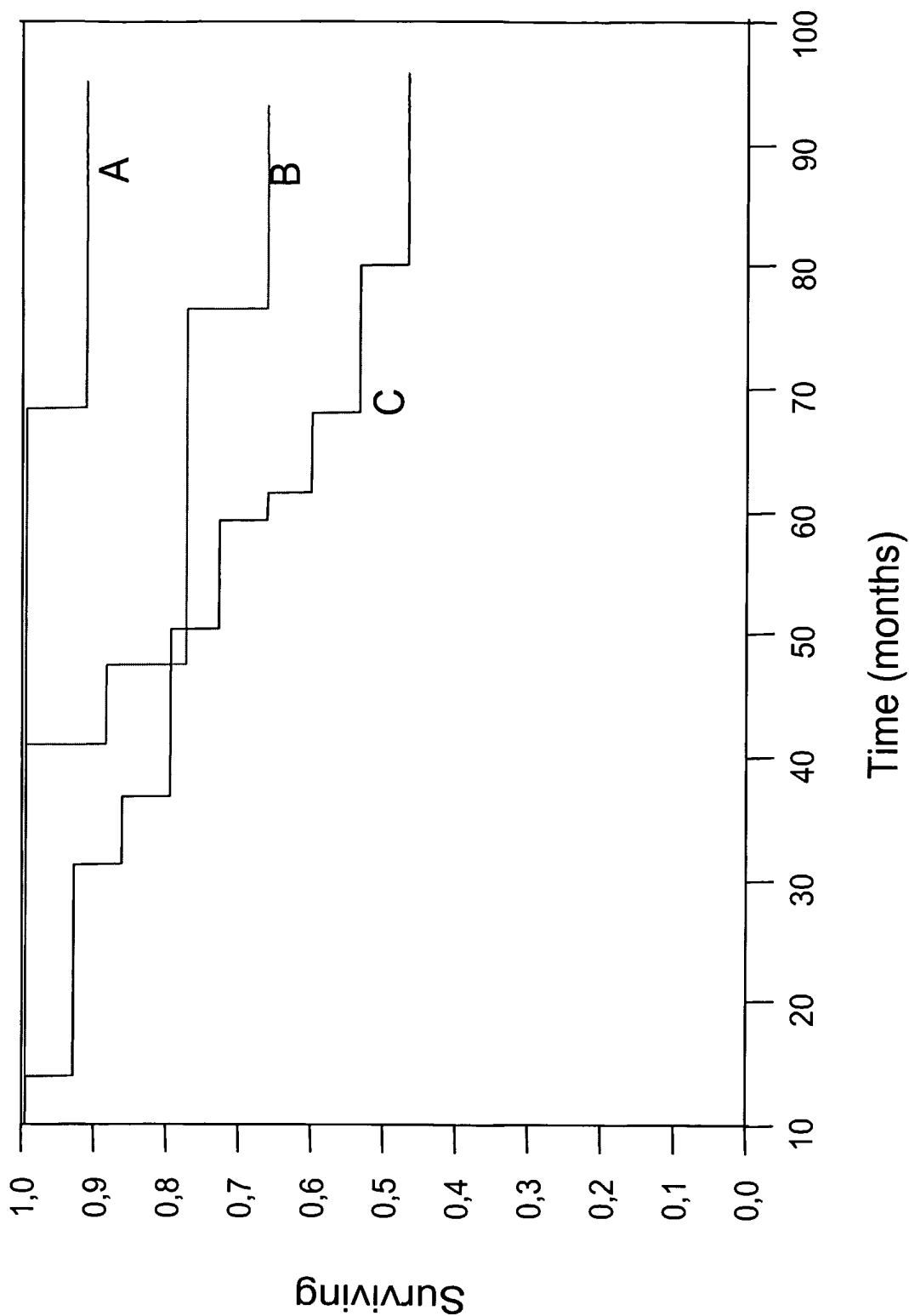


Figure 22. Patients with mild to moderate CHF (NYHA Class I-II)
A : N-proANP (1-98) <3292 fmol/ml AND BNP (RIA) <25 pg/ml
B : N-proANP (1-98) >3292 fmol/ml OR BNP (RIA) >25 pg/ml
C : N-proANP (1-98) >3292 fmol/ml AND BNP (RIA) >25 pg/ml

